

10/511320

AMENDMENTS TO THE CLAIMS

DT04 Rec'd PCT/PTO 15 OCT 2004

1. (Previously Presented) A substantially planar substrate for use in patch clamp analysis of the electrophysiological properties of a cell membrane comprising a glycocalyx, wherein the substrate comprises an aperture having a rim defining the aperture, the rim being adapted to form a gigaseal upon contact with the cell membrane, the rim protruding from the plane of the substrate to a height in excess of the thickness of the glycocalyx.

2. (Canceled)

3. (Currently Amended) A planar substrate according to Claim 1 wherein the rim protrudes from the plane of the substrate to a height of at least 20 nm above the surface of the planar substrate, preferably at least 30 nm, at least 40 nm, at least 50 nm, at least 60 nm, at least 70 nm, at least 80 nm, at least 90 nm or at least 100 nm.

4. (Currently Amended) A planar substrate according to Claim 1 ~~any one of the preceeding claims~~ wherein the width of the rim is in the range of 50 to 200 nm.

5. (Currently Amended) A planar substrate according to Claim 1 ~~any of the preceeding claims~~, in which the length (i.e., depth)

of the aperture is between 2 and 30  $\mu\text{m}$ , preferably between 2 and 20  $\mu\text{m}$ , 2 and 10  $\mu\text{m}$ , or 5 and 10  $\mu\text{m}$ .

6. (Currently Amended) A planar substrate according to Claim 1 ~~any of the preceding claims~~ wherein the diameter of the aperture is in the range of 0.5 to 2  $\mu\text{m}$ .

7. (Currently Amended) A planar substrate according to Claim 1 ~~any one of the preceding claims~~ wherein the rim extends substantially perpendicularly to the plane of the substrate.

8. (Currently Amended) A substrate according to Claim 1 ~~any one of Claims 1 to 5~~ wherein the rim forms an oblique angle with the plane of the substrate.

9. (Currently Amended) A substrate according to Claim 1 ~~any one of Claims 1 to 5~~ wherein the rim is substantially parallel to the plane of the substrate.

10. (Original) A substrate according to Claim 1 wherein the rim is defined by a mouth of the aperture, which mouth has a radius of curvature between 5 and 100 nm with an angle of 45 to 90 degrees.

11. (Currently Amended) A planar substrate according to Claim 1 ~~any of the preceding claims~~ wherein the substrate is made of silicon, plastics, pure silica or other glasses, such as quartz and Pyrex<sup>TM</sup>, or silica doped with one or more dopants selected from the group of Be, Mg, Ca, B, Al, Ga, Ge, N, P, As.

12. (Currently Amended) A planar substrate according to Claim 11 ~~10~~ wherein the substrate is made of silicon.

13. (Currently Amended) A substrate according to Claim 1 ~~any of the preceding claims~~ wherein the surface of the substrate and/or the walls of the aperture are coated with a second coating material.

14. (Currently Amended) A substrate according to Claim 13 ~~12~~ wherein the coating material is silicon, plastics, pure silica, other glasses such as quartz and Pyrex <sup>TM</sup>, silica doped with one or more dopants selected from the group of Be, Mg, Ca, B, Al, Ga, Ge, N, P, As, or oxides of the same.

15. (Currently Amended) A substrate according to Claim 11 ~~10~~ wherein the coating material is silicon oxide.

16. (Previously Presented) A method of making a substantially planar substrate for use in patch clamp analysis of the electrophysiological properties of a cell membrane comprising a glycocalyx, wherein the substrate comprises an aperture having a rim defining the aperture, the rim being adapted to form a gigaseal upon contact with the cell membrane, the method comprising the steps of

- (i) providing a substrate template;
- (ii) forming an aperture in the template; and
- (iii) forming a rim around the aperture such that the rim protrudes from the substrate to a height in excess of the thickness of the glycocalyx.

17. (Currently Amended) A method according to Claim 16 ~~15~~ wherein the substrate is manufactured using silicon micro fabrication technology.

18. (Currently Amended) A method according to Claim 16 ~~Claims 15 or 16~~ wherein step (ii) comprises forming an aperture by use of an inductively coupled plasma (ICP) deep reactive ion etch process.

19. (Currently Amended) A method according to Claim 16 ~~any one of Claims 15 to 17~~ further comprising the step of coating the surface of the substrate.

20. (Currently Amended) A method according to Claim 19 ~~18~~ wherein step (iii) is performed at the same time as coating the substrate.

21. (Currently Amended) A method according to Claim 19 ~~18~~ wherein step (iii) comprises an intermediate step of a directional and selective etching of the front side of the substrate causing a removal of a masking layer on the front side of the substrate, and further proceeding the prescribed protrusion distance into the underlying substrate.

22. (Currently Amended) A method according to Claim 19 ~~Claims 18, 19 or 20~~ wherein the coating is deposited by use of plasma enhanced chemical vapour deposition (PECVD) and/or by use of low pressure chemical vapour deposition (LPCVD).

23. (Currently Amended) A method according to Claim 22 ~~21~~ wherein the coating is deposited by use of plasma enhanced chemical vapour deposition (PECVD).

24. (Currently Amended) A method according to Claim 18 ~~17~~ wherein step (iii) comprises forming a rim from a deposited surface

coating by use of plasma enhanced chemical vapour deposition (PECVD).

25. (Currently Amended) A method for analysing the electrophysiological properties of a cell membrane comprising a glycocalyx, the method comprising the following steps:

(i) making a substantially planar substrate for use in patch clamp analysis of the electrophysiological properties of a cell membrane comprising a glycocalyx, wherein the substrate comprises an aperture having a rim defining the aperture, the rim being adapted to form a gigaseal upon contact with the cell membrane, the method comprising the steps of

(a) ~~(ii)~~ providing a substrate template;

(b) ~~(iii)~~ forming an aperture in the template; and

(c) ~~(iv)~~ forming a rim around the aperture such that the rim protrudes from the substrate to a height in excess of the thickness of the glycocalyx;—

(ii) ~~(v)~~ contacting the cell membrane with the rim of an aperture of the substrate such that a gigaseal is formed between the cell membrane and the substrate; and

(iii) ~~(vi)~~ measuring the electrophysiological properties of the cell membrane.

26. (Currently Amended) A kit for performing a method according to Claim 25 24, the kit comprising a substantially planar substrate for use in patch clamp analysis of the electrophysiological properties of a cell membrane comprising a glycocalyx, wherein the substrate comprises an aperture having a rim defining the aperture, the rim being adapted to form a gigaseal upon contact with the cell membrane, the rim protruding from the plane of the substrate to a height in excess of the thickness of the glycocalyx and one or more media or reagents for performing patch clamp studies.

27-29. (Canceled)